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## **METHODS, SYSTEMS AND PRODUCTS FOR DETERMINING DRUG WITHDRAWAL INTERVALS**

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this invention.

### **Field of the Invention**

15 This invention relates to methods and apparatus for determining a withdrawal  
interval for a new or off-label dosage of a compound, for consumable products derived  
from farm animals.

### **Background of the Invention**

20 Food safety is an important public health issue. One aspect of food safety is  
insuring that residue concentrations of drugs and other compounds in consumable  
products derived from food animals are at, or below, levels that are acceptable for human  
consumption. Programs for insuring such requirements are met are administered by  
various government regulatory agencies such as (in the United States) the Food and Drug  
25 Administration (FDA) and the United States Department of Agriculture (USDA).

In general, veterinary pharmaceutical products must meet standards of safety and  
efficacy, in much the same manner as human pharmaceutical products. In addition, it  
must be assured that an adequate time elapses between treatment of an animal and the  
harvesting of consumable products from that animal. Such times are termed "withdrawal  
30 times". From factors such as an acceptable daily intake of a given compound for human  
consumption, certain assumptions on the composition of the diet of a given population,

maximum residual levels (MRL; referred to as tolerance level in the United States) for various compounds in food products are determined. The withdrawal time is an indication of the time required, after treatment by or exposure to a particular compound, for the concentration of a compound (or byproduct of that compound) to reduce to a level at or below the tolerance in the tissue of interest (e.g., milk, meat). Once the withdrawal interval has elapsed, the tissue of interest can be harvested and used for human consumption. Food products are monitored for compliance with such tolerances, and the detection of levels in excess of acceptable tolerances can result in legal penalties.

Withdrawal intervals are currently determined empirically: a group of animals is administered a known drug at the dosage for which approval is sought, and animals within that group are sacrificed over time and the amount of the drug or residue measured in the tissue of interest. The withdrawal time is determined from the pharmacological data so obtained. A problem with this approach is that such studies require utilization of large groups of animals. An additional problem is that the study must be repeated, with an additional group of animals, when a new dosage is contemplated.

Veterinary and humane considerations can weigh in favor of using an approved drug for a sick animal at a dosage for which a withdrawal time is not known. In cases like this, either a best guess of a withdrawal time must be made, or an animal study must be conducted. If an insufficient withdrawal time is used and unacceptably high levels of the drug are detected in tissue derived from the treated animal, then legal liability under applicable food safety statutes may nevertheless attach.

Accordingly, there is a need for new techniques for determining withdrawal intervals in food animals.

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### **Summary of the Invention**

A first aspect of the invention is a method of estimating a withdrawal interval for an adjusted dose of a compound from a prior withdrawal time for a corresponding prior dose of that compound, corresponding half-life data and a tolerance concentration, for a tissue of interest. The method comprises selecting an adjusted dose for the compound for which a withdrawal interval is to be determined, and extrapolating a withdrawal interval from (a) the prior dose, (b) the prior withdrawal time, (c) the half-life data, and (d) the

tolerance concentration. An animal subject may then be administered the adjusted dosage of the compound, and then euthanized after the indicated withdrawal interval is completed and the tissue of interest harvested. Where the dose is administered prior to the extrapolating step (as may be the case with environmental contaminants consumed by food animals) the animal is euthanized and the tissue of interest harvested after the withdrawal interval from the date of administration of the compound has been completed.

In general, and as explained in greater detail below, the extrapolating step is carried out based on the slope of the line representing the tissue depletion for the compound in the tissue at the prior dose corresponding to the slope of the line representing the virtual depletion of the 99<sup>th</sup> percentile of the population of depleting animals for the compound in the tissue at the adjusted dose. Stated differently, the extrapolating step is carried out based on the depletion rate constant for the compound in the tissue at the prior dose corresponding to the virtual depletion rate constant representing the virtual depletion of the 99<sup>th</sup> percentile of the population of depleting animals for the compound in the tissue at the adjusted dose.

In carrying out the foregoing method, any of a variety of half-life data may be employed. The half-life data may be empirically determined data, such as an empirically determined effective residue half-life. The half-life data may be model pharmacokinetic data or an assumed or predetermined half-life multiplier. The tolerance concentration may be an official Tolerance (Tol) as determined by United States regulatory law, or may be a Maximum Residue Level (MRL) as determined by the laws of other jurisdictions. Where an MRL or Tol for one jurisdiction is used to determine a withdrawal interval for another jurisdiction in which a Tol or MRL has not been set, a provisional acceptable residue (PAR) or provisional acceptable tolerance (PAT) may be determined and used as the tolerance concentration in the foregoing, as explained in greater detail below.

When the half-life data is an empirically determined effective residue half-life, the step of extrapolating a withdrawal interval may be carried out by: determining a half-life multiplier from the first withdrawal time and the residue half-life; determining a concentration at time zero for the first dose from the tolerance concentration and the half-life multiplier; determining a concentration at time zero for the second dose from the first dose, the second dose, and the concentration at time zero for the first dose; and then

calculating the withdrawal interval from (a) the residue half-life, (b) the concentration at time zero for the second dose, and (c) the tolerance concentration. The calculating step may comprise executing the formula:

$$\text{WDI} = \text{ERH} \times \text{Ln}(\text{C}^{**}/\text{TOL}) \times 1.44$$

- 5 wherein: WDI=withdrawal interval; ERH=effective residue half-life; C\*\*= concentration at time zero for the second dose; and TOL=tolerance concentration.

When the half-life data is a half-life multiplier, the step of extrapolating a withdrawal interval may be carried out by: determining a residue half life from the first withdrawal time and the predetermined half-life multiplier; determining a concentration  
10 at time zero for the first dose from the tolerance concentration and the predetermined half-life multiplier; determining a concentration at time zero for the second dose from the first dose, the second dose, and the concentration at time zero for the first dose; and then calculating the withdrawal interval from (a) the residue half-life, (b) the concentration at time zero for the second dose, and (c) the tolerance concentration. The calculating step  
15 may comprise executing the formula:

$$\text{WDI} = \text{ERH} \times \text{Ln}(\text{C}^{**}/\text{TOL}) \times 1.44$$

wherein: WDI=withdrawal interval; ERH=effective residue half-life; C\*\*= concentration at time zero for the second dose; and TOL=tolerance concentration. The half life multiplier may be any suitable multiplier, e.g., a number from 1 to 10, and in one  
20 embodiment is 5.

The tolerance concentration may be a provisional acceptable residue (PAR) determined by the method comprising: providing an acceptable daily intake for the compound; partitioning the acceptable daily intake among tissues according to a set of partitioning instructions; and deriving the provisional acceptable residue for the tissue of  
25 interest from the partitioned acceptable daily intake.

When the compound is monitored in the tissue by monitoring a marker residue (i.e., one or more detectable compounds), the tolerance concentration may be a provisional acceptable tolerance (PAT) determined by the method comprising: providing an acceptable daily intake for the compound; partitioning the acceptable daily intake  
30 among tissues according to a set of partitioning instructions; deriving the provisional acceptable residue for the tissue of interest from the partitioned acceptable daily intake;

and determining a provisional acceptable tolerance from the provisional acceptable residue.

When the prior dose and the adjusted dose are carried out under different conditions (e.g., different formulations and/or different routes of administration), the  
5 extrapolating step may be preceded by the step of normalizing the conditions of the prior dose to the conditions of the adjusted dose.

In addition, the adjusted dose may be modified from the prior dose to account for species differences, disease differences, or both. The adjusted dose may be modified for disease differences including a change in clearance, a change in volume of distribution,  
10 and combinations thereof. This may be carried out based on known kinetic data. The adjusted dose may be modified for species differences based on known allometric data.

A second aspect of the present invention is a data processing system that carries out the foregoing methods.

A third aspect of the present invention is a computer program product for carrying  
15 out the foregoing methods and implementing the foregoing systems.

The foregoing and other objects and aspects of the present invention are explained in detail in the drawings herein and the specification set forth below, in which:

### **Brief Description of the Drawings**

20 **Figure 1** schematically illustrates an extrapolated withdrawal interval estimator system according to a preferred embodiment of the present invention.

**Figure 2** illustrates example tissue depletion curves employed in carrying out a preferred embodiment of the present invention.

**Figure 3** illustrates a system for determining a provisional acceptable residue  
25 (PAR) in accordance with a preferred embodiment of the present invention.

**Figure 4** is a graphical illustration of the conversion of a provisional acceptable residue to a provisional acceptable tolerance (PAT).

**Figure 5** illustrates operations performed by a dose adjuster system of a preferred embodiment of the present invention.

**Figure 6A** illustrates operations performed by a first withdrawal interval estimator of one embodiment of the present invention, employed when kinetic/regulatory data are not available.

5 **Figure 6B** illustrates operations performed by a second withdrawal interval estimator of an embodiment of the present invention, employed when kinetic/regulatory data are available.

**Figure 6C** illustrates operations performed by a withdrawal interval modifier of a preferred embodiment of the present invention.

10 **Figure 6D** illustrates operations performed by a withdrawal interval estimator of one embodiment of the present invention.

**Figure 6E** illustrates an example of a stochastic power function analysis of tissue depletion which would describe a drug with polyexponential or nonlinear kinetics.

#### **Detailed Description of the Preferred Embodiments**

15 This invention may be embodied in many different forms and should not be construed as limiting to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

20 It will be understood that elements of the invention can be implemented by general and/or special purpose hardware-based system that perform the specified functions or steps, or by combinations of general and/or special purpose hardware and software instructions.

25 These program instructions may be provided to a processor to produce a machine, such that the instructions that execute on the processor create means for implementing the functions specified in the illustrations. The computer program instructions may be executed by a processor to cause a series of operational steps to be performed by the processor to produce a computer implemented process such that the instructions which execute on the processor provide steps for implementing the functions specified in the illustrations. Keyboards, touch-screens, mouse/screen pointer combinations and the like  
30 all provide means for accepting selection of a particular parameter by the systems described herein. Accordingly, the figures herein support combinations of means for

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performing the specified functions, combinations of steps for performing the specified functions and program instruction means for performing the specified functions.

The following terms and abbreviations are used herein. Some of these terms (and other terms used herein) are in accordance with standard regulatory usage by the applicable regulatory agency (*e.g.*, the United States Department of Agriculture (USDA); the United States Food and Drug Administration (FDA); and the counterpart regulatory agencies of other jurisdictions); others are defined herein for the purpose of explaining and carrying out the instant invention:

**ADI:** Acceptable Daily Intake (typically expressed as mg or ug of compound/60kg person/day). Obtainable from U.S. or foreign data files. Generally, the ADI is calculated by dividing the NOEL obtained in the toxicology study with the most appropriate species by a safety factor.

**C\*:** Estimated (typically determined at the 99<sup>th</sup> percentile) C<sub>T</sub> for label dose.

**C\*\*:** Estimated (typically determined at the 99<sup>th</sup> percentile) C<sub>T</sub> for extralabel dose.

**CF:** Correction Factor to adjust half-life label-dose for disease or altered physiology of extralabel conditions.

**C<sub>T</sub>:** Mean concentration in tissue at time zero which reflects the administered working label dose.

**E\_Dose:** Extralabel dose.

**ERH:** Effective Residue Half-Life. The half-life related to the slope of the virtual line which defines the upper bound of the applicable confidence interval (typically the upper bound of a 95% confidence interval around the 99<sup>th</sup> percentile) for ETH (operationally defined as the line connecting WDT<sub>99</sub> and C\*).

**ETH:** Effective Tissue Half-life. Mean (50<sup>th</sup> percentile) tissue depletion half life.

**EW\_Dose:** Extra-label Working Dose. Normalized by dosage regimen.

**EWE:** Extrapolated Withdrawal-interval Estimator algorithm.

**Hlife:** Half-Life.

**HLM:** Half-Life Multiplier. Number of half-lives to reach tolerance/MRL level.

**IPPIA:** Iterative Population Pharmacokinetics Interval Adjuster.

**L-Dose:** Label-Dose.

**LOQ:** Limit of Quantitation. The lowest concentration that can be reproducibly detected by an analytical method. The LOQ is the Tol/MRL in some jurisdictions that have “zero-residue” policies in effect.

**LW\_Dose:** Label Working Dose. Normalized by dosage regimen.

5 **MRL:** Maximum Residue Levels (typically expressed as mg/kg). This is similar to tolerance derivation in the U.S.

**Marker residue:** The compound, degradation product(s) of that compound, or combination thereof in a tissue used to determine the withdrawal time for that tissue for the compound administered to the animal, and from which the marker residue is  
10 derived.

**NOEL:** The no observed effect level. Defined as the greatest concentration or amount of an agent, found by study or observation, that causes no detectable, usually adverse, alteration of morphology, functional capacity, growth, development or lifespan of the target (e.g., the subject of a toxicology study).

15 **PARs:** Provisional Acceptable Residues (typically expressed as ppm). Derivation of PARs is similar to FDA-CVM derivation of SC as defined below.

**PATs:** Provisional acceptable tolerances (typically expressed as ppm). Derivation of PATs is similar to FDA-CVM derivation of tolerance as defined below.

**RVIS:** Residue Violation Information System. USDA regulatory residue violation  
20 database.

**SC:** Safe Concentration (typically expressed as ppm). The concentration of residues in tissues considered safe for human consumption. One definition of SC is as follows:

$$25 \quad SC = \frac{ADI \text{ (mg/kg/day)} \times 60 \text{ kg}}{\text{grams consumed/day}}$$

**TOL:** Tolerance level (typically expressed as either ppm or ppb). The concentration of the marker residue in the target tissue when the concentration of the total residue of toxicological concern is equal to the permitted safe concentration in the last  
30 tissue to deplete to its safe concentration.

**TRIMS:** Tissue Residue Information Management System. FDA monitoring residue database.



**WDI:** Withdrawal Interval. The EWE estimated withdrawal period. Defined to avoid confusion with WDT.

**WDT:** The approved label-dose Withdrawal time, as approved by the appropriate regulatory agency. Withdrawal time for FDA approved or foreign approved drugs.

5       The present invention may be employed with any farm, or food, animal, including but not limited to mammalian avian and aquatic (e.g., fish, crustacean) species such as cows, pigs, lamb, rabbit, chicken, turkey, ostrich, catfish, tilapia, trout, catfish, Mahi-Mahi, crayfish, shrimp, lobster, etc. Thus, examples of tissues that may be employed in the present invention include but are not limited to muscle (or meat), liver, kidney, fat,  
10       egg, and milk.

      The present invention may be employed with any of a variety of compounds, including drugs, pesticides, and environmental contaminants, which may be administered to animals intentionally or unintentionally by any route of administration including, but not limited to, topical application to the skin or hide, inhalation, oral administration (e.g.,  
15       a drug tablet, powder, granules or capsule; by the eating of food contaminated with a pesticide or environmental contaminant), parenteral injection (e.g., subcutaneous injection, intraperitoneal injection; intravenous injection). Illustrative examples of compounds that may be employed in connection with the present invention include, but are not limited to:

20       ampicillin, amoxacillin, fenthion, famphur, phosmet, chlorpyrifos, flunixin meglumine, trichlorofon, doxycycline, lindane, methoxychlor, permethrin, acepromazine, detomidine, ketamine, xylazine, yohimbine, aspirin, dipyrone, ketoprofen, phenylbutazone, cephalixin, penicillin G, novobiocin, cloxacillin, erythromycin, tylosin, florfenicol, pyrantel, ciprofloxacin, doramectin, enrofloxacin, milbemycin oxime,  
25       danofloxacin, sarafloxacin, abamectin, albenzadole, azaperone, benzylpenicillin, carazolol, carbadox, ceftiofur sodium, chlortetracycline, oxytetracycline and tetracycline, clenbuterol, closantel, cypermethrin, alpha-cypermethrin, dexamethasone, diclazuril, dihydrostreptomycin and streptomycin, diminazene, doramectin, estradiol-17beta, febantel, fenbendazole, oxfendazole, flubendazole, gentamicin, isometamidium  
30       ivermectin, levamisole, moxidectin neomicin, oxfendazole, progesterone, spectinomycin, spiramycin, all sulfonamides (e.g., sulfadimidine), streptomycin, testosterone,

thiamphenicol, thiabendazole, tilmicosin, trenbolone acetate, triclabendazole, zeranol, etc. In general, many compounds that are used in the present invention have first order clearance kinetics in the tissue of interest

Drugs and compounds may be administered to the animal by any route, including  
5 but not limited to topical, oral, parenteral (including intramuscular, intravenous, intraarterial, subcutaneous, and intraperitoneal injection), transdermal, rectal, nasal, inhalation, etc.

One embodiment of the present invention is an extrapolated withdrawal-interval estimator (EWE), schematically illustrated in **Figure 1**. This embodiment estimates a  
10 withdrawal interval (WDI) for an extralabel use of drugs in the United States. This process is consistent with US food safety standards and with the policies of extralabel drug use expounded in the AMDUCA regulations promulgated by FDA in 1997. It will be appreciated that the same process can be used to estimate an extralabel use of a drug in a jurisdiction other than the United States, or to predict an appropriate WDI for use of a  
15 drug approved in one jurisdiction (e.g., a US drug) for export to another jurisdiction to meet the latter's food safety standards.

In overview, the process and apparatus of **Figure 1** obtains its data from two primary sources of information: the approved labeled reference product and pharmacokinetic data. In brief overview, the withdrawal interval estimator **6** (which may  
20 be either embodiment **6a** or **6b** below) receives a provisional acceptable residue, or PAR, from the PAR module (see blocks **3**, **3'**). Where the dose or route of administration differs between the approved dose and the extralabel dose a dose adjustment module **5** may be employed to normalize dosage, and where species is changed from approved dose to extralabel dose an interspecies allometric scaling module **6c** may be employed to  
25 normalize data. As field data for the new dosage is accumulated, the estimate may be modified by the use of the iterative population pharmacokinetic interval adjuster module (see blocks **6d**, **6d'**).

As shown in **Figure 2**, a primary assumption in the present invention is that the label reference product contains a withdrawal time that statistically insures with 95%  
30 confidence that approximately 99% of the population of animals treated with this product under label dosage conditions will have tissue concentrations at slaughter below the

established tolerances or maximum residue levels (MRLs; line 21) established as safe for that country. Thus any data extracted from this information is in reality an estimate of the upper band of the confidence interval for depletion (C\*; line 22) in the worst case scenario in the rate limiting tissue (defined either as having the slowest depletion or as most sensitive from a food safety perspective).

Note that a confidence interval higher or lower than 95% can be used (e.g., a confidence interval of 90, 95, or 99 percent or more) and that a population percentile higher or lower than 99% can be employed (e.g., a percentile of 90, 95, 99, or 99.5 percent or more). In general, different parameters will still yield a line as in Figure 2 having essentially the same slope, or will yield essentially the same depletion rate constant. Accordingly, it will be appreciated that it is the slope or rate constant that is significant to the operation of the instant invention, and not the assignment of a particular percentile or confidence interval.

Turning to **Figure 3**, since, in the present example, the drug is to be used in the United States, when US approved labels are not available, data from other jurisdictions are the best source of information for food safety available. However, the foreign withdrawal time obtained from the label may not be appropriate for application in the United States, since different factors are used to extrapolate toxicology results (e.g. NOAELs) to estimates of human safety (e.g. Acceptable Daily Intake - ADI). However, it is assumed that foreign ADIs are the best available source of safety information which would be appropriate for US standards if the ADI were adjusted for US/foreign differences in safety factors and tissue-specific food consumption factors (see blocks 31, 32). Thus the EWE method and apparatus illustrated in **Figure 1** takes foreign ADIs and in the PARs module (schematically illustrated in **Figure 3**), uses US assumptions procedures to partition the ADI over target tissues in accordance with partitioning instructions (Figure 3, blocks 33, 33a, 34, 34a, 35, 35a, 35b) to arrive at PARs for each tissue (see blocks 36a, 36b, 36c, 36d). If US tolerances or safe levels are available, this step is not necessary (see blocks 31a, 31b). This step is also necessary for extralabel use in minor species and in this case would be applied using US ADIs as the input data. Finally, the PARs is further adjusted by a consumption factor since it is a safety

parameter reflecting total residues, and compared against the original  $ADI_{US}$  to identify potential health risks (blocks 37, 38, 39).

In some cases one or more marker residues are monitored rather than the drug or compound administered. As graphically illustrated in **Figure 4**, the PAR is adjusted to the marker residue, which is monitored, and a Provisional Acceptable Tolerance (PAT) is calculated for each tissue to be used as the target concentration against which a WDI is determined.

**Figure 5** illustrates the dose adjuster module (block 5 of Figure 1) from data entry (block 50) to application of the WDI estimator or modifier routines (blocks 6a, 6b, 6c, 6d). This module normalizes label dosage to extralabel conditions by calculating working doses which reflect the amount of drug in the body at the time of the last drug administration. This is achieved by defining working doses (which normalizes or reflects the normalized amount of drug in the tissue at the end of a dosage regimen) for both the label {LW\_Dose} and extralabel {EW\_Dose} dosage conditions. The drug formulations selected to compare will have similar dosage formulations (e.g. rapid acting, long-acting) to insure that the rate limiting kinetic process is similar in both cases (e.g. systemic clearance versus absorption limited disposition). For example, if both label and extralabel usage are single doses, then the adjustment is simply based on a ratio of doses. However, if the label dose is multiple and the extralabel dose single, then standard clinical pharmacokinetic techniques are used to estimate working doses (blocks 51, 52, 53a, 53b, 53c, 54a, 54b, 54c, 500, 501).

In the **Figure 5**, equations based on monoexponential disposition (steady state and non-steady state) are used, although if more precise multi-exponential or nonlinear models are available to describe tissue-sequestered or metabolized drugs, then they could be incorporated (block 50a). Similarly, differences in bioavailability (F) are allowed for and used if good information is available. If not, then F is set to 1.0 which is a conservative assumption from a food safety perspective. The result of this module are LW\_Dose and EW\_Dose, the latter being used to calculate the WDI.

The Withdrawal Interval Estimator 6 is a central feature of the EWE illustrated in Figure 1. At this point, one has an EW\_Dose and a target tissue concentration (MRL, Tol or PAT). The problem now is to use the label dose information to extract disposition

parameters that reflect a level of statistical confidence appropriate to regulatory policies. To mathematically define a tissue depletion profile, two attributes are required: a slope reflecting rate of tissue decay, and an intercept reflecting drug concentration at some defined time. The regulatory data provides the latter in terms of the target tolerance, or MRL, which occurs for the entire population at the withdrawal time. However, the label working dose also gives similar information if the volume of distribution and/or plasma to tissue ratio is known. It must be stressed that this phase of the analysis is using the reference label data only to extract this depletion information and is not being used for any food safety extrapolations since other factors not applicable to the US regulatory environment (e.g. Good Veterinary Practices used in Europe) may modify the MRL relative to ADI-criteria alone. The assumption is that the statistics governing the establishment of an approved withdrawal time will insure that any parameters extrapolated from regulatory data will have a conservative statistical estimate built in. The overall strategy is outlined in **Figure 2**, taken together with the various embodiments of the withdrawal interval estimators and modifier exemplified by **Figures 6A, 6B, 6C, and 6D**.

**Figure 2** defines two sets of intercepts and slope-parameters. Any parameter obtained from the literature (e.g. pharmacokinetic) or from raw data in regulatory submissions, is defined as representing mean population estimates (50th percentile). The zero-time intercept is thus  $C_T$  and the slope of line **20** is  $-0.693/ETH$ , where ETH is the Effective Tissue Half-life. Since most tissue depletion data is governed by first-order pharmacokinetic decay, the concept of half-life is operative; otherwise, another model is used (see below).

If data is available suggesting that tissue decay is non-linear (e.g. due to metabolism), then an analytical equation for decay is written or a stochastic power-function regression as outlined in the WDI modifier illustrated in **601a** and **Figures 6D-6E** is used. If a specific pharmacokinetic model for the drug or chemical is known, then this model is directly used in estimator **601** (Fig 6D block **601**) and a WDI is calculated. This approach would describe the tissue depletion profile for a drug having any degree of complexity (e.g., nonlinear metabolism or tissue binding). However, for most drugs and

dosages used in veterinary medicine, or contaminating compounds, first-order tissue depletion kinetics will be applicable.

In contrast, data extracted from label information reflects parameters estimated for the upper bound of a statistical confidence interval. In the US, this is the 99<sup>th</sup> percentile estimated with an  $p < 0.05$ . In data from other jurisdictions, such a regression approach is not used but the withdrawal time approved is assumed to reflect a similar degree of statistical confidence. The only assumption made is that the regulatory experimental design assures that the vast majority of animals slaughtered at the prescribed withdrawal time will be below the target tolerance or MRL **21**. It is irrelevant whether this hypothesis is tested using parametric estimates of the tissue depletion regression function or non-parametric point estimates of a withdrawal time. The result is that the data obtained from a label package represents, for our purposes, the 99th percentile of the  $C_T$  and ETH, which we term  $C^*$ , and is bounded by the line **22** defined by the virtual ERH (Effective Residue Half-life).

It will be appreciated that the extrapolating step can be carried out in any manner, and the corresponding extrapolating means implemented in any manner, such as by graphic computation, exponential computation, trigonometric computation, etc.

The EWE essentially takes a number of independent approaches to estimate these four parameters. Viewed in a different light, this approach is actually estimating the statistical properties (mean, variance) of  $C_T$  and ETH. In the final implementation of this process (**Figure 6D; 602**), this data is used in this manner and the output will simply be the 99th percentile of the disposition function to calculate a  $WDI_{99}$  (**603**). Population pharmacokinetics and Bayesian estimation algorithms allow this process to be implemented in a manner that may be validated with field observations (e.g., feedback data in the form of tissue samples from animals after slaughter).

The problem is to determine how long it takes for drug to deplete from  $C_T$  to tolerance. At this stage, the estimators employed in **Figure 6A** and **Figure 6B** are used to independently estimate these parameters and then calculate WDIs for each process. As will be seen, in **Figure 6a** the half life multiplier is assumed, while in **Figure 6b** a  $C_T$  and ETH are obtained from kinetic or regulatory data **62**. Otherwise, like components of **Figures 6a and 6b** are assigned like numbers. Both begin with input of an adjusted dose

**61a, 61b.** In block **64a, 64b**, Tol. is used if the label data is US and MRL is used in its place if label data is foreign; in block **66a, 66b**, Tol. is used if label data is US and PAT is used if the treatment is not approved in this country, or if the compound is an environmental contaminant (e.g., heptachlor, chlordane).

5        A critical factor calculated in this analysis is the half-life multiplier (HLM) which computes the number of ETHs or ERHs required to get from  $C_T$  to tolerance. In **Figure 6a** the initial estimate is the default kinetic assumption of 5 (97% eliminated). However, as more drug data is processed through EWE, this default parameter will be more accurately estimated and probably will become drug-class dependent at some point in the  
10       future. As shown in **Figure 6d**, by decomposing the withdrawal time into its constitutive pharmacokinetic parameters, disease or physiological states (e.g. age) may be accounted for by using literature to determine their influence on ETH or  $C_T$  (blocks **61d, 601, 601a, 602, 602a, 603**).

      Disease effects are partitioned across changes in volume of distribution and  
15       clearance to make our estimate more robust. (**Figure 6C**, blocks **661, 662, 663, 664a, 664b, 665, 666**) Similarly, when dose extrapolations are made across species, allometric exponents relating half-lives between species can be applied to ETH/ERH to adjust for the species being treated (**Figure 6C**, blocks **671, 672, 673, 674, 675**).

      Returning to **Figure 2**, the final step of the EWE process is to calculate the actual  
20       99th percentile starting tissue concentration ( $C^{**}$ ) which reflects the actual extralabel dosage used. This is obtained by adjusting the reference label  $C^*$  by the working dosages calculated above (LW\_Dose, EW\_Dose). The problem now is merely to determining how long is required for drug to deplete from  $C^{**}$  to target tolerance or PAT, that is the WDI, when depletion is governed by ERH. (see lines **23, 24** in **Figure 2**). The above series of  
25       calculations are conducted on each tissue for both safety (PAR) and pharmacokinetic (ETH,  $C_T$ ) endpoints. Multiple WDIs are determined and the longest one will be selected for recommendation. This process thus provides the regulatory statistical assurances and extrapolates the resulting  $WDI_{99}$ .

      The final complication which could occur involves administering compound  
30       drugs or the same drug by multiple routes (e.g. intrammary and systemic). For different drugs, the longest WDI will be selected. For multiple routes, one approach is simply to

use the principle of superposition and add the tissue depletion curves to obtain a combined WDI, implemented as a multiple drug/route adjuster module (**Figure 1; 5a**).

**Feedback and Validation:** As can be appreciated from this process, there are a number of assumptions which may not hold for many drugs. The EWE process exemplified above is formulated as an iterative procedure which allows feedback from previous analyses or actual field observations to improve the estimate. For example, the more accurate the pharmacokinetic data, the better the WDI estimates in the processes of **Figure 6B** and **Figure 6C**. Similarly, the output from this process has regulatory and food safety impact. Thus, the EWE can be executed in a population pharmacokinetic environment where each parameter being estimated is linked to independent field or clinical data to improve the estimate. An additional strength of this approach is that as such data is modeled, the nature of the statistical distribution around the WDI will be obtained.

An optimal source of such data is available in the USDA-RVIS and FDA-TRIMS surveillance and monitoring databases. By inputting this data into EWE, the statistical confidence around EWE's WDI estimates will be achieved. This can be analyzed using both parametric and nonparametric statistical techniques. Use of this data also suggests that a primary use of the EWE process would be to design the monitoring and validation sampling strategies using estimated WDIs to determine under what circumstances and field use conditions residues would be most likely to occur. The primary difference between the parametric approach and the nonparametric approach is that the parametric model actually estimates ETH and  $C_T$  directly and determines the nature of the statistical distribution operative. In contrast, the nonparametric model only allows as input concentrations that are above tolerance/PAT (+) or below (-). What is being tested is simply whether  $WDI_{99}$  can differentiate the breakpoint between + and -. This is equivalent to conducting a slaughter test (as has been done in some countries) and taking the withdrawal time as that which occurs when all animals are below MRL. (Note that a recent series of papers by Concordet and Toutain {*J. Vet. Pharmacol. Therap.* **20**: 374-379; 380-386, 1997} derive nonparametric statistical tests to accomplish this). It is not suggested herein whether a parametric regression or nonparametric approach is superior to simply validate a WDI. In contrast, the implementation of these tests would ultimately



improve the underlying model used to calculate the WDI. Other sources of data could include the individual animal results from a screening assay; producer group's quality assurances programs where recommended WDIs could be validated under various dose conditions. Similarly, data submitted from industry research could improve these models.

- 5 A final scenario is that a small sample of veterinarians requesting WDIs from FARAD would be requested to send back samples for validation.

**Implementation:** The invention is executed in a Visual-Basic database program that has regulatory and pharmacokinetic data modules. Specific files for input of data can include the following:

- 10 1. A file of approved label drugs with dosages, indications and withdrawal times for US and Foreign countries.
2. A file tabulating pharmacokinetic data based on "experiments" resulting from analysis of data for a specific dose, route, matrix, and disease in a species.
3. A file of US approved tissue tolerances and safe concentrations;
- 15 Foreign drug approvals; Validated EWE-calculated PATs.
4. A file of screening tests, official FSIS/FDA assays with LOQs and statistics which can be directly implemented in the population model.
5. A tabulation of US and Foreign ADIs.
6. A summary datafile storing interspecies allometric coefficients and
- 20 mean pharmacokinetic data.
7. A bridging database which obtains EWE usable dose, concentration-time data extracted from databases such as RVIS/TRIMS.
8. A file containing the results of validated WDI estimates.

In conclusion, the present invention is useful for extending the estimation of

25 validated WDIs for extralabel drug use to be used as approved WDTs under the new flexible labeling guidelines since a defensible statistical inference procedure is available. The same approach can be used to establish approved WDTs for minor species uses. EWE can also be used to extrapolate WDIs based on foreign MRLs or LOQs in those cases where "zero-residue" policies are operative, for use by US producers exporting to

30 those countries. In the context of a global FARAD, this process can be used to cross-extrapolate between any two countries as long as PARs for the country of use could be

adequately defined. The invention can also be used as a development tool to design experiments to support formal regulatory data packages or help establish WDTs for “flexible-label” products. Finally, the invention can be used for approval of any withdrawal time submission in lieu of repeated slaughter studies.

5           The present invention is explained in greater detail in the following non-limiting Examples.

### EXAMPLE 1

#### Computation of a WDI from Regulatory Data

10           A simulation was conducted to demonstrate the similarity between the WDI predictions obtained by the EWE method (**Procedure B**), and the WDT determined by the FDA official method. The FDA method to establish withdrawal times is based on a statistical Tolerance Limit procedure, and has been published in the *Guideline for Establishing a Withdrawal Period* (Center for Veterinary Medicine). This guideline  
15           contains a real example on how the FDA establishes WDT. The instructions contained in this example were used to carry out the simulation described herein.

**FDA Tolerance Limit Procedure.** Drug tissue concentrations at slaughter time from twenty-five animals are obtained. target tissues from animals are sampled in groups of five at five different sampling times according to the depletion characteristics of the  
20           drug. A regression analysis is then conducted and a series of tests are performed to verify the validity of the assumptions implied by the statistical method. The first step is to assess whether any data point falls on the linear part of the depletion curve for the phase of the curve closest to the MRL or Tolerance. All data points are supposed to be above the validated LOQ of the analytical method for any statistical inference to be acceptable.  
25           The next step is to assure the constancy of variances for each datapoint (homoscedasticity). Bartlett’s test is used to check on this point, although the guideline states that other methods can be used instead. Finally, an ANOVA test is conducted to verify the log-linearity of the regression line.

          When the regression line is shown to conform to the statistical assumptions, a set  
30           of calculations is conducted to establish the tolerance limit for a “candidate” withdrawal time, according to the method of Owen. This initial WDT is established arbitrarily, and if

for that candidate withdrawal time the tolerance limit exceeds the permitted Tolerance, then the calculation is repeated adding one day to the previous candidate WDT. This process is repeated until the tolerance limit falls under the official Tolerance for a particular WDT. This last candidate is established as the official WDT.

5        **Simulations.** The following pharmacokinetic and dosing conditions were established for the initial simulations: The label dose was 400 mg, administered as a single intravenous bolus dose. The elimination rate in the target tissue was  $0.1155 \text{ day}^{-1}$ , the volume of distribution of the central compartment was 11.67 liters. The tissue plasma ratio was 2.7. The label tolerance was 17 ppm. The variance of the  
10       concentration, expressed in logarithmic scale, was 0.071. With these conditions 1000 individuals (5 datapoints each) were simulated (5000 datapoints). Out of all these, 25 datapoints were selected randomly (5 datapoints per time). The WDT established by the FDA method for this group of animals using the Tolerance Limit method above was 23 days. By using the elimination constant ( $K_{el}$ , or slope of the regression line) as the only  
15       input into the EWE process a WDI of 22.95 was predicted for the same group of animals, dosing, and tolerance conditions (see Table 1).

This example demonstrates that the EWE process sets a WDI similar to the official WDT under the same dosing conditions, when the slope of the regression curve is known (from literature or regulatory data).

20

## EXAMPLE 2

### **Computation of an Extra-Label WDI from Regulatory Data**

This example was conducted to assess how well the EWE process would predict a WDI for an extralabel use. This simulation, called EWESIN\_2, was set with the  
25       following conditions: dose was increased to 1000 mg and the extralabel Tolerance (different of the label MRL, if foreign) was 12 ppm. The same slope was used as in Example 1, since that would be the procedure followed in practice: obtain that information from label, regulatory or literature data and apply to all calculations. The WDI was set to 34.41. In order to validate this result we simulated again 1000 animals  
30       and 5,000 datapoints using the same level of variance, the same Pk parameters and the new dose of 1000 mg. We selected randomly 25 datapoints from 25 animals and applied

the Tolerance Limit procedure aiming at a new Tolerance: 12 ppm. Again, the result was 34 days. See Table 2. The biologically insignificant differences between EWE-predicted WDIs and the Tolerance Limit Procedure WDT is based on random errors inherent to sampling 25 data points from a population of 5,000 simulated data points.

5

### EXAMPLE 3

#### Computation of an Extra-Label WDI from Regulatory Data with Disease Affecting Clearance

Another simulation was conducted with the same 1000 mg and 12 ppm of tolerance as before, with the half-life arbitrarily increased 1.73-fold. Assuming the increase in half-life was due entirely to a decrease in clearance, the estimate of WDI obtained by the EWE process was 58.5 days (see Table 3). Assuming monoexponential decay of drug in the target tissue, an increase of half-life that is totally due to clearance can be formulated as:

$$\begin{aligned} \text{Kel}^{**} &= \text{Kel}_{\text{label}} / \text{CF} \\ \text{Vd}^{**} &= \text{Dose}_{\text{label}} / \text{C}^{*} \end{aligned}$$

where  $\text{Kel}^{**}$  is the elimination rate constant in disease conditions,  $\text{Kel}_{\text{label}}$  is the elimination rate constant in normal conditions, CF is the ratio of half-lives, or correction factor,  $\text{Vd}^{**}$  is the virtual volume of distribution in tissue (including the statistical confidence limit) under disease conditions, and  $\text{C}^{*}$  has been defined previously. We again simulated 1000 tissue depletion curves with 5000 datapoints and selected 25 randomly. This simulation was called EWESIM\_3. The FDA's Tolerance Limit procedure was applied to these data and the result was a WDT of 61 days (Table 3).

25

### EXAMPLE 4

#### Computation of an Extra-Label WDI from Regulatory Data with Disease Affecting Volume of Distribution

This example, called EWESIN\_4, is essentially the same as Example 3, except that it was assumed that the increase in half-life was due entirely to an increase in volume. The WDI obtained by EWE was 50.32 days (see Table 4). The Tolerance Limit

30

procedure established 49 days as the WDT based on 25 randomly selected datapoints from a simulated dataset consisting of 5,000 samples (Table 4).

The effect of volume change on half-life for this example can be formulated as:

$$Kel^{**}=Kel_{label}/CF$$

$$Vd^{**}=(Dose_{label}/C^{*}) \times CF$$

where  $Kel^{**}$  is the elimination rate constant in disease conditions,  $Kel_{label}$  is the elimination rate constant in normal conditions, CF is the ratio of half-lives, or correction factor,  $Vd^{**}$  is the virtual volume of distribution in tissue (including the statistical confidence limit) under disease conditions, and  $C^{*}$  has been defined previously.

#### EXAMPLE 4

##### **Computation of an Extra-Label WDI from Regulatory Data with Disease Affecting Both Clearance and Volume of Distribution**

Finally the same type of simulation as in Examples 3 and 4 was accomplished but this time, the increase in half life by a factor of 1.7 was partially due to an increase in  $Vd$  and partially due to a decrease in clearance (fifth-fifty). This example, called EWESIN\_5, estimated a WDI of 53.87 days while the Tolerance Limit Procedure established the WDT at 54 days. Results are given in Table 5. This simulation was formulated as:

$$Kel^{**}=Kel_{label}/CF$$

$$Vd^{**}=Dose_{label}/C^{*}(1+((CF-1)/2))$$

where  $Kel^{**}$  is the elimination rate constant in disease conditions,  $Kel_{label}$  is the elimination rate constant in normal conditions, CF is the ratio of half-lives, or correction factor,  $Vd^{**}$  is the virtual volume of distribution in tissue (including the statistical confidence limit) under disease conditions, and  $C^{*}$  has been defined previously.

Note that concomitant changes in volume of distribution and clearance by factors different from 50% of the initial value can also be allowed by simply partitioning the total 100% of change in the value of half-life differentially between clearance and volume of distribution

The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention has been explained above primarily with respect to drugs,

but it may also be used for other compounds administered to animals deliberately or inadvertently, such as pesticides and environmental contaminants. In addition, the invention has largely been explained for practice within the United States using the regulatory nomenclature of the United States, but the invention is not limited to any  
5 particular regulatory nomenclature and can be practiced in other jurisdictions in accordance with the regulatory nomenclature of those jurisdictions.

Table 1. Dose Equals 400 mg

EWESIN_1. Computation of WDT and WDI from regulatory (FDA) data.								
1. Computation of WDT and WDI from regulatory (label) data.								
WDT	CONC (ppm)	Ln Conc		WDT	Avg Conc	Avg Ln C	Variance	Ln Var
3	62.3451	4.133		3	62.631	4.129	0.021	-3.861
3	49.2054	3.896		7	40.791	3.695	0.034	-3.377
3	67.3542	4.210		12	22.924	3.097	0.088	-2.434
3	72.3654	4.282		20	9.235	2.192	0.077	-2.567
3	61.875	4.125		30	3.409	1.176	0.133	-2.017
7	31.462	3.449		14.4	27.797788	2.858	0.071	-2.851
7	40.432	3.700						
7	47.1254	3.853	FDA Tolerance Limit Method calculations:					
7	35.9694	3.583	a=	4.444	BDSS=	1502	u=	1.656
7	48.9658	3.891	b=	-0.110	d=	7.788	k=	10.927
12	16.204	2.785	s <sup>2</sup> =	0.062	z=	2.326	Tol <sub>lim</sub> =	2.726
12	24.2671	3.189	s=	0.250	t <sub>ave</sub> =	14.400	exp(t <sub>lim</sub> )=	15.279
12	16.8401	2.824	df=	23.000	[...] <sup>1/2</sup> =	0.299	Tol=	17.000
12	24.1904	3.186	n=	25.000	[...]=	0.089		
12	33.1178	3.500	MDW=	14.400	n=	0.043	WDT=	23.00
20	7.1254	1.964	EWE Process calculations:					
20	8.6325	2.156						
20	6.69325	1.901	LW_dose=	400.00	Co=	31.51	ERH=	6.29
20	10.8457	2.384	EW_dose=	400.00	TPR=	2.70	WDT <sub>50</sub> =	14.60
20	12.877	2.555	MRL=	17	CT <sub>label</sub> =	85.07	HLM <sub>50</sub> =	2.32
30	4.6457	1.536	Tol=	17	V <sub>c</sub> =	12.69	HLM <sub>99</sub> =	3.65
30	3.50124	1.253	L_Hlife=	6.29	Kel=	0.11	C*=	213.90
30	2.57400	0.945	R_Hlife=	1.00	ETH=	6.29	C**=	213.90
30	4.3658	1.474	E_Hlife=	6.29	CF=	1.00	WDI=	22.95
30	1.956	0.671						
2. Bartlett's Test for Homogeneity of the Variances								
(s <sub>i</sub> ) <sup>2</sup> =Variance of Ln concentration in i <sup>th</sup> group								
g=Number of Withdrawal groups = 5								
f=Common # of df for variance estimate = 4								
Time	(s <sub>i</sub> )	Ln(s <sub>i</sub> ) <sup>2</sup>	f <sub>i</sub>	f <sub>i</sub> s <sub>i</sub> <sup>2</sup>	f <sub>i</sub> Ln s <sub>i</sub> <sup>2</sup>	1/f <sub>i</sub> -1		
3	0.021	-3.861	4	0.0841984	-15.443495	0.333333		
7	0.034	-3.377	4	0.1366323	-13.507025	0.333333		
12	0.088	-2.434	4	0.350707	-9.7363939	0.333333		
20	0.077	-2.567	4	0.3069551	-10.269392	0.333333		
30	0.133	-2.017	4	0.5324635	-8.0661409	0.333333		
SUMS=	0.353	-14.256	20	1.4109564	-57.022447	1.666667		
M=	3.99315665							
C=	1.00694444							
X <sup>2</sup> <sub>g-1</sub> =	3.96561764							
M/C=	3.96561764							
P=	>0.25							

Table 2. Dose equals 1000 mg.

EWESIN_2: Simulation for WDI determination from WDT data.								
1. Tolerance limit and EWE procedures. No disease effect. Extralabel dose 3 times label dose.								
WDT	CONC (ppm)	Ln Conc		WDT	Avg Conc	Avg Ln C	Variance	Ln Var
3	169.37	5.132		3	161.368	5.076	0.021	-3.879
3	171.37	5.144		7	101.281	4.605	0.032	-3.429
3	187.33	5.233		12	57.831	4.038	0.049	-3.011
3	149.42	5.007		20	23.127	3.089	0.129	-2.051
3	129.37	4.863		30	7.737	2.000	0.133	-2.178
7	114.52	4.741		14.4	70.26864	3.761	0.069	-2.910
7	91.29	4.514	FDA Tolerance Limit Method calculations:					
7	76.97	4.343						
7	103.66	4.641	a=	5.406	BDSS=	1502	u=	1.656
7	119.97	4.787	b=	-0.114	d=	4.278	k=	6.586
12	77.07	4.345	s <sup>2</sup> =	0.060	z=	2.326	Tol <sub>lim</sub> =	2.401
12	43.22	3.766	s=	0.245	t <sub>ave</sub> =	14.400	exp(t <sub>lim</sub> )=	11.037
12	50.27	3.917	df=	23.000	[...] <sup>1/2</sup> =	0.544	Tol=	12.000
12	63.69	4.154	n=	25.000	[...]=	0.296		23.000
12	54.91	4.006	MDW=	14.000	n=	0.043	WDT=	34.00
20	21.12	3.050						
20	19.87	2.989	EWE Process calculations:				V <sub>d</sub> **=	1.87
20	23.93	3.175	LW_dose=		Co=		ERH=	6.29
20	37.02	3.612	EW_dose=		TPR=		WDT <sub>50</sub> =	14.59
20	13.70	2.617	MRL=		CT <sub>label</sub> =		HLM <sub>50</sub> =	2.32
30	6.06	1.801	Tol=		V <sub>c</sub> =		HLM <sub>99</sub> =	3.65
30	5.32	1.672	L_Hlife=		Kel=		C*=	213.90
30	9.94	2.297	R_Hlife=		ETH=		C**=	534.76
30	11.29	2.424	E_Hlife=		CF=		WDI=	34.41
30	6.08	1.804						
2. Bartlett's Test for Homogeneity of the Variances								
(s <sub>i</sub> ) <sup>2</sup> =Variance of Ln concentration in i <sup>th</sup> group								
g=Number of Withdrawal groups = 5								
f=Common # of df for variance estimate = 4								
Time	(s <sub>i</sub> )	Ln(s <sub>i</sub> ) <sup>2</sup>	f <sub>i</sub>	f <sub>i</sub> s <sub>i</sub> <sup>2</sup>	f <sub>i</sub> Ln s <sub>i</sub> <sup>2</sup>	1/f <sub>i</sub> -1		
3	0.021	-3.879	4	0.082655	-15.51747	0.333333		
7	0.032	-3.429	4	0.129618	-13.71784	0.333333		
12	0.049	-3.011	4	0.196914	-12.04514	0.333333		
20	0.129	-2.051	4	0.51454	-8.203107	0.333333		
30	0.113	-2.178	4	0.453215	-8.710733	0.333333		
SUMS=	0.344	-14.549	20	1.376942	-58.19428	1.666667		
M=	4.67693786							
C=	1.00694444							
X <sup>2</sup> <sub>g-1</sub> =	4.64468312							
M/C=	4.64468312							
P=	>0.25							



Table 3. Altered Clearance

EWESIN_3: simulation for WDI determination from WDT data. Impaired clearance.								
1. Tolerance limit and EWE procedures. Disease affects clearance.								
WDT	CONC (ppm)	Ln Conc		WDT	Avg Conc	Avg Ln C	Variance	Ln Var
3	230.15	5.439		3	189.528	5.232	0.031	-3.479
3	171.41	5.144		7	138.461	4.916	0.035	-3.340
3	176.37	5.173		12	106.177	4.647	0.047	-3.049
3	151.25	5.019		20	62.713	4.103	0.095	-2.355
3	218.46	5.387		30	30.804	3.378	0.133	-2.015
7	167.35	5.120		14.4	105.53668	4.455	0.068	-2.848
7	131.66	4.880	FDA Tolerance Limit Method calculations:					
7	122.89	4.811						
7	107.57	4.678	a=	5.430	BDSS=	1502	u=	1.656
7	162.85	5.093	b=	-0.068	d=	1.909	k=	3.807
12	112.55	4.723	s <sup>2</sup> =	0.060	z=	2.326	Tol <sub>lim</sub> =	2.439
12	129.35	4.863	s=	0.245	t <sub>ave</sub> =	14.400	exp(t <sub>lim</sub> )=	11.460
12	74.96	4.317	df=	23.000	[...] <sup>1/2</sup> =	1.219	Tol=	12
12	119.55	4.784	n=	25.000	[...]=	1.486	L_WDT=	23.00
12	94.48	4.548	MDW=	14.400	n=	0.043	WDT=	61.00
20	83.24	4.422	EWE Process Calculations:					
20	38.54	3.652						
20	51.10	3.934					Kel**=	0.06
20	69.46	4.241	LW_dose=	400.00	Co=	31.51	Vd**=	1.87
20	71.23	4.266	EW_dose=	1000.00	TPR=	2.70	ERH=	10.70
30	29.68	3.390	MRL=	17	CT <sub>label</sub> =	85.07	WDT <sub>50</sub> =	14.59
30	37.25	3.618	Tol=	12	V <sub>c</sub> =	12.70	HLM <sub>50</sub> =	2.32
30	42.90	3.759	L_Hlife=	6.29	Kel=	0.11	HLM <sub>99</sub> =	3.65
30	16.54	2.806	R_Hlife=	1.70	ETH=	6.29	C**=	213.90
30	27.66	3.320	E_Hlife=	10.70	CF=	1.70	C**=	534.76
							WDI=	58.49
2. Bartlett's Test for Homogeneity of the Variances								
(s <sub>i</sub> ) <sup>2</sup> =Variance of Ln concentration in i <sup>th</sup> group								
g=Number of Withdrawal groups = 5								
f=Common # of df for variance estimate = 4								
Time	(s <sub>i</sub> )	Ln(s <sub>i</sub> ) <sup>2</sup>	f <sub>i</sub>	f <sub>i</sub> s <sub>i</sub> <sup>2</sup>	f <sub>i</sub> Ln s <sub>i</sub> <sup>2</sup>	1/f <sub>i</sub> -1		
3	0.031	-3.479	4	0.123308	-13.91745	0.333333		
7	0.035	-3.340	4	0.141763	-13.35956	0.333333		
12	0.047	-3.049	4	0.189656	-12.19535	0.333333		
20	0.095	-2.355	4	0.37944	-9.421417	0.333333		
30	0.133	-2.015	4	0.5333359	-8.059422	0.333333		
SUMS=	0.342	-14.238	20	1.367526	-56.9632	1.666667		
M=	3.2986171							
C=	1.0069444							
X <sup>2</sup> <sub>g-1</sub> =	3.275868							
M/C=	3.275868							
P=	>0.25							

Table 4. Altered Volume

EWESIN_4: simulation for WDI determination from WDT data. Increased volume.								
1. Tolerance limit and EWE procedures. Disease affects volume of distribution.								
WDT	CONC (ppm)	Ln Conc		WDT	Avg Conc	Avg Ln C	Variance	Ln Var
3	122.531	4.808		3	105.761	4.654	0.018	-4.032
3	95.2303	4.556		7	90.159	4.486	0.041	-3.190
3	104.649	4.651		12	58.204	4.019	0.112	-2.188
3	116.997	4.762		20	34.026	3.493	0.097	-2.334
3	89.3995	4.493		30	15.987	2.739	0.089	-2.423
7	64.36	4.164		14.4	60.82741	3.878	0.071	-2.833
7	86.5168	4.460	FDA Tolerance Limit Method calculations:					
7	102.709	4.632						
7	108.254	4.684	a=	4.919	BDSS=	1502	u=	1.656
7	88.9545	4.488	b=	-0.072	d=	2.543	k=	4.535
12	69.257	4.238	s <sup>2</sup> =	0.064	z=	2.326	Tol <sub>lim</sub> =	2.428
12	84.58	4.438	s=	0.253	t <sub>ave</sub> =	14.400	exp(t <sub>lim</sub> )=	11.338
12	42.865	3.758	df=	23.000	[...] <sup>1/2</sup> =	0.915	Tol=	12
12	37.445	3.623	n=	25.000	[...]=	0.837	L_WDT=	23.00
12	56.8724	4.041	MDW=	14.400	n=	0.043	WDT=	49.00
20	32.956	3.495	EWE Process Calculations:					
20	19.245	2.957						
20	41.254	3.720						
20	36.932	3.609	LW_dose=	400.00	Co=	31.51	ERH=	10.70
20	39.7448	3.682	EW_dose=	1000.00	TPR=	2.70	WDT <sub>50</sub> =	14.59
30	18.054	2.893	MRL=	17	CT <sub>label</sub> =	85.07	HLM <sub>50</sub> =	2.32
30	20.118	3.002	Tol=	12	V <sub>c</sub> =	12.70	HLM <sub>99</sub> =	3.65
30	13.845	2.628	L_Hlife=	6.29	Kel=	0.11	C*=	213.90
30	9.658	2.268	R_Hlife=	1.70	ETH=	6.29	C**=	314.56
30	18.258	2.905	E_Hlife=	10.70	CF=	1.70	WDI=	50.32
2. Bartlett's Test for Homogeneity of the Variances								
(s <sub>i</sub> ) <sup>2</sup> =Variance of Ln concentration in i <sup>th</sup> group								
g=Number of Withdrawal groups = 5								
f=Common # of df for variance estimate = 4								
Time	(s <sub>i</sub> )	Ln(s <sub>i</sub> ) <sup>2</sup>	f <sub>i</sub>	f <sub>i</sub> s <sub>i</sub> <sup>2</sup>	f <sub>i</sub> Ln s <sub>i</sub> <sup>2</sup>	1/f <sub>i</sub> -1		
3	0.018	-4.032	4	0.070965	-16.1274	0.333333		
7	0.041	-3.190	4	0.164711	-12.7594	0.333333		
12	0.112	-2.188	4	0.448675	-8.751	0.333333		
20	0.097	-2.334	4	0.387822	-9.33401	0.333333		
30	0.089	-2.432	4	0.3546	-9.69223	0.333333		
SUMS=	0.357	-14.166	20	1.426774	-56.6641	1.666667		
M=	3.8577769							
C=	1.0069444							
X <sup>2</sup> <sub>g-1</sub> =	3.8311716							
M/C=	3.8311716							
P=	>0.25							

Table 5. Altered Clearance and Altered Volume

EWESIN\_5: simulation for WDI determination from label data. 50% increased Vd, 50% decreased clearance.

1. Tolerance limit and EWE procedures. Disease affects equally clearance and volume of distribution.

WDT	CONC (ppm)	Ln Conc		WDT	Avg Conc	Avg Ln C	Variance	Ln Var
3	135.213	4.907		3	136.896	4.912	0.018	-4.007
3	119.54	4.784		7	109.783	4.686	0.033	-3.425
3	121.568	4.800		12	76.948	4.341	0.070	-2.664
3	166.955	5.118		20	44.546	3.757	0.102	-2.285
3	141.203	4.950		30	21.876	3.033	0.132	-2.028
7	132.25	4.885		14.4	78.40978	4.146	0.071	-2.882
7	106,528	4.668	FDA Tolerance Limit Method calculations:					
7	86.525	4.460						
7	127.258	4.846	a=	5.159	BDSS=	1502	u=	1.656
7	96.354	4.568	b=	-0.070	d=	2.234	k=	4.179
12	107.25	4.675	s <sup>2</sup> =	0.062	z=	2.326	Tol <sub>lim</sub> =	2.444
12	93.67	4.540	s=	0.249	t <sub>ave</sub> =	14.400	exp(t <sub>lim</sub> )=	11.517
12	61.25	4.115	df=	23.000	[...] <sup>1/2</sup> =	1.041	Tol=	12
12	74.205	4.307	n=	25.000	[...]=	1.084	L_WDT=	23.00
12	58.365	4.067	MDW=	14.400	n=	0.043	WDT=	54.00
20	51.895	3.949	EWE Process Calculations:					
20	29.657	3.390						
20	48.58	3.883						
20	61.05	4.112	LW_dose=	400.00	Co=	31.51	Kel**=	0.06
20	31.547	3.451	EW_dose=	1000.00	TPR=	2.70	Vd**=	2.52
30	16.3521	2.794	MRL=	17	CT <sub>label</sub> =	85.07	ERH=	10.70
30	20.9217	3.041	Tol=	12	V <sub>c</sub> =	12.70	WDT <sub>50</sub> =	14.59
30	33.17	3.502	L_Hlife=	6.29	Kel=	0.11	HLM <sub>50</sub> =	2.32
30	25.7208	3.237	R_Hlife=	1.70	ETH=	6.29	HLM <sub>99</sub> =	3.65
30	12.2169	2.581	E_Hlife=	10.70	CF=	1.70	C*=	213.90
							C**=	396.12
							WDI=	53.87

2. Bartlett's Test for Homogeneity of the Variances

(s <sub>i</sub> ) <sup>2</sup> =Variance of Ln concentration in i <sup>th</sup> group						
g=Number of Withdrawal groups = 5						
f=Common # of df for variance estimate = 4						
Time	(s <sub>i</sub> )	Ln(s <sub>i</sub> ) <sup>2</sup>	f <sub>i</sub>	f <sub>i</sub> s <sub>i</sub> <sup>2</sup>	f <sub>i</sub> Ln s <sub>i</sub> <sup>2</sup>	1/f <sub>i</sub> -1
3	0.018	-4.007	4	0.072719	-16.02976	0.333333
7	0.033	-3.425	4	0.130255	-13.69823	0.333333
12	0.070	-2.664	4	0.278668	-10.65611	0.333333
20	0.102	-2.285	4	0.40694	-9.141534	0.333333
30	0.132	-2.028	4	0.52642	-8.111798	0.333333
SUMS=	0.354	-14.409	20	1.415003	-57.63744	1.666667
M=	4.6654252					
C=	1.0069444					
X <sup>2</sup> <sub>g-1</sub> =	4.6332499					
M/C=	4.6332499					
P=	>0.25					